PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

To:
BASSETT, Richard, S.
Eric Potter Clarkson
Park View House
58 The Ropewalk
Nottingham NG1 5DD
ROYAUME-UNI

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 03 August 2000 (03.08.00)	**IMPORTANT NOTICE**
Applicant's or agent's file reference DELF/P22390PC	
International application No. PCT/GB00/00257	**International filing date** (day/month/year) 31 January 2000 (31.01.00)
Priority date (day/month/year) 30 January 1999 (30.01.99)	
Applicant DELTA BIOTECHNOLOGY LIMITED et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,
   NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   03 August 2000 (03.08.00) under No. WO 00/44772

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The Internati nal Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
ERIC POTTER CLARKSON
Attn. BASSETT, Richard S
Park View House
58 The Ropewalk
Nottingham NG1 5DD
UNITED KINGDOM

Applicant's or agent's file reference DELF/P22390PC	Date of mailing *(day/month/year)* 31/08/2000 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/GB 00/00257	international filing date *(day/month/year)* 31/01/2000
Applicant DELTA BIOTECHNOLOGY LIMITED et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mireille Claudepierre

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
DELF/P22390PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00257	31/01/2000	30/01/1999
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant DELTA BIOTECHNOLOGY LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 14 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23/08/2000	03.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mundel, C Telephone No. +49 89 2399 7314

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00257

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-73 as originally filed

Claims, No.:

1-53 as originally filed

Drawings, sheets:

1/17-17/17 as originally filed

Sequence listing part of the description, pages:

1-6, filed with the letter of 16.03.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-19, 21-22, 29-31, 33, 37-40, 43-44, 50-51 and 53
	No:	Claims	1-7, 20, 23-28, 32, 34-36, 41-42, 45-49 and 52
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-53
Industrial applicability (IA)	Yes:	Claims	1-53
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II
Priority

The priority document of the present application was not available at the time where this preliminary opinion has been drafted. The present analysis is based on the hypothesis that all the claims have a priority right corresponding to the date of filing of the priority document (30.01.99)

Re Item IV
Lack of unity of invention

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The International Preliminary Examination Authority (IPEA) agrees with the ISA advices that the present application lacks unity and identifies the following groups of inventions in the international application :

A. Claims 1-7 refer to a process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

B. Claims 8-52 refer to a process for purifying an albumin solution which involves a succession of chromatographic steps.

C. Claim 53 refers to a DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

Methods for the production of human serum albumin are known in the prior art. WO9404687 describes a method for the production of recombinant proteins (including HSA), which involves the use of a fungal cell with at least a reduced capacity for O-mannosylation due to genetic manipulation of one or more genes involved in that process (the construction of a mutant in PMT1 is given as example). Another method for producing heterologous proteins involving the use of fungal cells which have lost -at

least in part- their capacity for O-mannosylation is described in WO946873; in this case the cells are not genetically modified in specific genes which take part in the O-mannosylation process, but are the result of random mutagenesis and subsequent screening.

In the light of the prior art, the problem of the underlying application can be defined as the provision of further methods to obtain human serum albumin.

The solution as described and claimed in this application can be summarized as follows:

(i) Process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

(ii) Process for purifying an albumin solution which involves a succession of chromatographic steps.

(iii) DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

In the view of the fact that the methods for producing albumin, the methods for purifying the same and recombinant DNA molecules encoding albumin are already disclosed in the prior art, due to essential difference in the nature of the three problems and their corresponding solutions and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical feature common to these solutions, the IPEA agrees with the ISA advices that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rules 13.1 PCT. Consequently, the present application lacks unity and the different groups identified above represent independent inventions.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Reference is made to the following documents :**

D1: WO 97 31947 A (DELTA BIOTECHNOLOGY LTD ;GOODEY ANDREW ROBERT (GB); SLEEP DARRELL) 4 September 1997 (1997-09-04)

D2: WO 94 04687 A (STRAHL BOLSINGER SABINE ;TANNER WIDMAR (DE); FLEER REINHARD (FR);) 3 March 1994 (1994-03-03) cited in the application

2. **Lack of novelty and inventive step; articles 33(2) and 33(3) PCT.**

Invention I :

Claims 1-7 of the present application refer to a process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

The document D2 discloses fungal cells carrying specific modifications which cause them to exhibit a **reduced capacity for O-glycosylating** homologous and/or heterologous proteins and the use of these cells as host cells for producing high yields of recombinant products (Abstract). The problems due to an undesirable O-glycosylation on fungal derived recombinant products was well-known from the authors of D2 (p. 3). The fungal cells of D2 can be chosen from filamentous fungi and yeasts (p. 4, last paragraph and p. 5, lines 1-2). The modified fungal cells carry genetic modifications in at least one gene whose expression product is involved in the attachment of a mannosyl residue to the hydroxyl group of seryl or threonyl amino acids and more particularly the gene encoding the Dol-P-Man:Protein (Ser/Thr) Mannosyl Transferase : **PMT1** (p. 7, lines 1-21). The process for preparing the modified fungal cells is such that the modifications are **stable during segregation and/or non-reverting and/or non-leaky** (p. 5, lines 30-35). D2 also refers to a process for the production of recombinant products including **human serum albumin** using such modified

fungal cells (p. 10, lines 4-10 and line 16-17). Finally, D2 exemplifies the preparation of an S. cerevisiae cell deficient in O-glycosylation activity (Example 6, p. 23-25 and example 7, p. 25-28).

The IPEA is the opinion that the process disclosed in D2 can be applied to every volume of culture medium and that the pH range disclosed in claim 1 of the present application is a standard pH range for yeast cultures. Therefore, the subject-matter of claims 1-7 can not be considered as novel or inventive in view of D2 (article 33(2) and 33(3) PCT).

Invention II :

Claims 8-52 refer to a process for purifying an albumin solution which involves a succession of chromatographic steps.

A. Novelty; article 33(2) PCT.

1. The document D1 discloses a process for the preparation of albumin which has extremely low levels of or is essentially free of colorants, metal ions, human proteins, host proteins, fragments of albumin, polymers or aggregates of albumin and viruses, and which is essentially non-glycated, relatively high in free thiol and with an intact C-terminus (Abstract, lines 1-6). The process comprises passing albumin (preferably expressed and secreted by transformed yeast) through positive mode cation exchange chromatography and then positive mode anion exchange chromatography (Abstract, lines 6-9). D1 also states that other steps like ultrafiltration, gel permeation chromatography, affinity chromatography binding the albumin using blue dyes or chromatography affinity binding contaminants like aminophenylboronic acid resin can be used (Abstract, lines 9-13). The elution of albumin with a compound having affinity for albumin, from a material having no specific affinity for albumin is also disclosed (Abstract, lines 13-15). D1 discloses the conditioning of the albumin solution with octanoate - an albumin stabiliser - to a final concentration of 1-10 mM and a pH about 4.0-5.0 (p. 3, lines 18-21).

Example 2 of D1 illustrate a process of purification of the albumin which comprises the steps of : cation exchange, affinity chromatography, ultrafiltration, gel permeation (with ultrafiltration of recycle fraction) and anion exchange. In this example, the cation exchange chromatography uses commercial cation exchange matrix such as SP-Sepharose (which comprises sulfopropyl substituents) (p. 21, first paragraph). It is also stated that the pH of the albumin solution used for cation exchange chromatography is adjusted to 4.3-4.8 (p. 16, lines 12-13) and that octanoate is added to the solution to a final concentration of 1-10 mM (p. 16, lines 9- 11). In example 2, before the step of anion exchange chromatography, the pooled recycle fraction is concentrated to a retentate concentration of 20- 120 g/L albumin (p. 24, lines 22-23). The anion exchange chromatography uses a matrix like DEAE-Spherodex or DEAE-cellulose (comprising immobilised dialkylaminoalkyl substituents as anion exchangers) (p. 25, lines 11-13)
Example 6 of D1 illustrate a variation of the process of example 2 or 4. In this example, the eluate from the cation exchange column was diluted to below 10 $mS.cm^{-1}$ (p. 34, lines 27-29) and directly loaded on the anion exchange chromatography column.
Example 3 of D1 refers to the formulation of purified albumin into a final product.

Since there is no mention in most of the claims of the present application that the anion exchange chromatography should follow directly the cation exchange chromatography, the subject-matter of claims 20, 23-28, 32, 34- 36, 41-42, 47-49 and 52 can not be considered as novel in the sense of article 33(2) PCT.

Moreover, since the step of gel permeation chromatography involve the use of a solution having a pH 5.4-5.6, this step can be considered as a process for reducing the level of nickel ions in an albumin solution according to claims 45 and 46. Therefore, claims 45 and 46 lack novelty (article 33(2) PCT).

2. The subject-matter of claims 8-19, 21-22, 29-31, 33, 37-40, 43-44 et 50-51 has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, claims 8-19, 21-22, 29-31, 33, 37-40, 43-44 et 50-51 are considered as novel in the sense of article 33(2) PCT.

B. Inventive step; article 33(3) PCT.

The document D1 is considered as the most relevant document for the evaluation of the inventiveness of claims 8-52 (see point A above for the content).

D1 also discloses the purification steps of :

(i) Positive affinity chromatography using an immobilised albumin-specific dye such as Cibacron Blue type of dye (p. 4, lines 10-19) which can be used to purify the albumin with respect to the 45 kDa N-terminal albumin fragment (p. 22 to 23).

(ii) Ultrafiltration (p.4, lines 21-31 and p. 23-24).

(iii) Gel filtration in order to purify albumin with respect to yeast antigens, pigments and dimerised albumin (p. 24).

(iv) Negative affinity chromatography with respect to albumin on a matrix with immobilised aminophenylboronate in order to remove glycoconjugates such as glycoproteins and glycolipids and poly-, oligo- and monosaccharides (Example 7, p. 35-37).

D1 states that the step involving immobilised phenylboronate may be used earlier in the process (p. 39, example 8).

The document D1 which concerns purification of recombinant human albumin discloses all the purification steps used in the present application and the result obtained after purification are similar to those obtained with the purification process disclosed in the present application, i.e. :

(i) A negligible level of glycation of the purified recombinant human albumin (p. 40, line 24-26).

(ii) A low molecular weight contaminants total peak area for albumin purified by the process of D1 which is of less than 10% of that for

human serum albumin (p. 44, lines 22-24).

(iii) Recombinant human albumin purified in accordance with the process disclosed in D1 has a stable and full length carboxy-terminus whereas HSA previously available from commercial sources appears to be heterogeneous by comparison (p. 48, lines 20-22).

(iv) A free thiol value of 0.85-0.9 mole SH/ mole rHA for albumin purified according to the process of D1 (p. 50, lines 26-27).

(v) Lower level of Al, Fe, Cu, Mg, Zn and Mn in the product of the process according to D1 than in the albumin of the prior art.

(vi) Differences in the medium and long chain fatty acid content of the albumin purified according to D1 with respect to commercial HSA (p. 57, lines 27-32 to p. 59, line 4).

(vii) Lower absorbance at 350, 403 and 500 nm than number of commercially available HSA preparations (p. 59, lines 20-21).

In view of the teaching of D1 which discloses all the important steps for the purification of human albumin used in the present application, the IPEA considers that the fact to combine the different purification steps in another order than the order disclosed in D1 or the fact to add well-known purification steps like cation or anion exchange chromatography run in negative mode with respect to albumin can not be considered as inventive. Moreover, the results of the purification process of D1 seem to be similar to the results obtained with the process of the present application. The IPEA also considers that the adjustments in the pH values, concentration and conductivity of the different solutions, which are necessary to perform the process, are current practice for the skilled person and do not imply any inventive activity.

Therefore, claims 8-44 and 47-52 can not be considered as involving an inventive step in the sense of article 33(3) PCT.

In the argumentation concerning inventive step, the applicant should demonstrate what could be the advantage of a process according to the present application over the process disclosed in the document D1.

Invention III :

Claim 53 refers to a DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

Novelty; article 33(2) PCT.

The subject-matter of claim 53 has never been disclosed in the documents cited in the ISR. Therefore, claim 53 is considered as novel in the sense of article 33(2) PCT.

Inventive step; article 33(3) PCT.

The document D1 is considered as the closest prior art for the evaluation of the inventiveness of claim 53.

D1 discloses the fact that yeast may be transformed with an expression plasmid containing an expression cassette comprising a yeast promoter, a sequence encoding a secretion leader, the HSA (human serum albumin) coding sequence and a transcription terminator (p. 8, lines 14-26).

In 1999 (the present application claims the priority date of 30.01.99), it was well- known for the skilled person that, in order to reduce the problem of read-through from the ribosomes in an expression system, the number of in frame translation stop codons at the 3' end of a coding sequence could be increased.
Therefore, the IPEA considers that claim 53 does not involve any inventive step (article 33(3) PCT).

Re Item VIII

Certain observations on the international application

Lack of clarity; article 6 PCT.

Inventions I, II and III :

1. Claim 1 of the present application lacks clarity for the following reasons :
   (i) The genetic modification of the cell is only defined by the fact that it causes the cell to have a reduced capacity of mannosylation, i.e. by the result to be achieved by said genetic modification.
   According to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.7 : "The area defined by the claims must be as precise as the invention allows. As a general rule, claims which attempt to define the invention, or a feature thereof, by a result to be achieved should be objected to".
   This remark also applies to claims 2-4.
   (ii) The sequences of the recombinant albumin or the recombinant albumin coding sequence are not given what renders the scope of the claim unclear.
   This remark also applies to claim 53.
   (iii) The attention of the applicant is drawn to the fact that, in the present application, the only fungal cell disclosed is a Saccharomyces cerevisiae cell mutated in the PMT1 gene. Moreover, all the fungal cells disclosed in the patent application WO 94/04687 cited in the present application are fungal cells having a mutation in a PMT gene. Therefore, the IPEA considers that the use of fungal cells having a genetic modification other than a mutation of a PMT gene is not supported by the description of the present application (article 5 PCT in combination with article 6 PCT).

2. In claim 5, the term "preferably" is used. The attention of the applicant is drawn to the fact that, according to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.6 : expressions, like "preferably", "for example", "such as" or "more particularly" should be regarded "as having no limiting effect on the scope of a claim; that is to say, the feature following any such expression should be regarded as entirely optional".

This remark also applies to claims 6, 7, 9, 10, 12, 14, 15, 24, 25, 26, 30, 31, 33-40, 42, 45, 49 and 53.

3. Claim 8 of the present application lacks clarity because there is no reference to what the "first albumin solution" should exactly be.

4. In claim 12, the use of the vague term "about" renders the scope of the claim unclear.

5. Claims 26 precise that the albumin solution has an octanoate ion concentration of 2-15 mM. The attention of the applicant is drawn to the fact that there no mention in the preceding claims that the albumin solution should contain an octanoate ion.

6. In claim 38, the compound present in the buffer used to elute the albumin from the anion exchanger is only characterized by the fact that it has a specific affinity for albumin, i.e. by the result to be achieved by said buffer, what should be avoided (see point VIII-1(i)).

7. In claim 42, It is not clear what is precisely meant by "primary separation" and "centrate conditioning".

8. In claim 48, there is no mention to which kind of "derivation" is meant what renders the scope of said claim unclear.

14

PATENT COOPERATION TREATY

PCT

REC'D 07 MAY 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
DELF/P22390PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/00257	31/01/2000	30/01/1999
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant DELTA BIOTECHNOLOGY LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 14 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23/08/2000	03.05.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mundel, C Telephone No. +49 89 2399 7314

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/00257

I. Basis of th rep rt

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-73 as originally filed

Claims, No.:

1-53 as originally filed

Drawings, sheets:

1/17-17/17 as originally filed

Sequence listing part of the description, pages:

1-6, filed with the letter of 16.03.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8-19, 21-22, 29-31, 33, 37-40, 43-44, 50-51 and 53
	No:	Claims	1-7, 20, 23-28, 32, 34-36, 41-42, 45-49 and 52
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-53
Industrial applicability (IA)	Yes:	Claims	1-53
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re It m II
Priority

The priority document of the present application was not available at the time where this preliminary opinion has been drafted. The present analysis is based on the hypothesis that all the claims have a priority right corresponding to the date of filing of the priority document (30.01.99)

Re Item IV
Lack of unity of invention

According to **Rule 13 PCT** an application must relate only to one invention or to a group of inventions so linked as to form a **single inventive concept**, i.e. having at least one common technical feature defining a contribution over the known prior art.

The International Preliminary Examination Authority (IPEA) agrees with the ISA advices that the present application lacks unity and identifies the following groups of inventions in the international application :

A. Claims 1-7 refer to a process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

B. Claims 8-52 refer to a process for purifying an albumin solution which involves a succession of chromatographic steps.

C. Claim 53 refers to a DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

Methods for the production of human serum albumin are known in the prior art. WO9404687 describes a method for the production of recombinant proteins (including HSA), which involves the use of a fungal cell with at least a reduced capacity for O-mannosylation due to genetic manipulation of one or more genes involved in that process (the construction of a mutant in PMT1 is given as example). Another method for producing heterologous proteins involving the use of fungal cells which have lost -at

least in part- their capacity for O-mannosylation is described in WO946873; in this case the cells are not genetically modified in specific genes which take part in the O-mannosylation process, but are the result of random mutagenesis and subsequent screening.

In the light of the prior art, the problem of the underlying application can be defined as the provision of further methods to obtain human serum albumin.

The solution as described and claimed in this application can be summarized as follows:

(i) Process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

(ii) Process for purifying an albumin solution which involves a succession of chromatographic steps.

(iii) DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

In the view of the fact that the methods for producing albumin, the methods for purifying the same and recombinant DNA molecules encoding albumin are already disclosed in the prior art, due to essential difference in the nature of the three problems and their corresponding solutions and due to the fact that no other technical feature can be distinguished which, in the light of the prior art, could be regarded as special technical feature common to these solutions, the IPEA agrees with the ISA advices that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of Rules 13.1 PCT. Consequently, the present application lacks unity and the different groups identified above represent independent inventions.

R It m V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Reference is made to the following documents :**

   D1: WO 97 31947 A (DELTA BIOTECHNOLOGY LTD ;GOODEY ANDREW ROBERT (GB); SLEEP DARRELL) 4 September 1997 (1997-09-04)

   D2: WO 94 04687 A (STRAHL BOLSINGER SABINE ;TANNER WIDMAR (DE); FLEER REINHARD (FR);) 3 March 1994 (1994-03-03) cited in the application

2. **Lack of novelty and inventive step; articles 33(2) and 33(3) PCT.**

   Invention I :

   Claims 1-7 of the present application refer to a process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

   The document D2 discloses fungal cells carrying specific modifications which cause them to exhibit a **reduced capacity for O-glycosylating** homologous and/or heterologous proteins and the use of these cells as host cells for producing high yields of recombinant products (Abstract). The problems due to an undesirable O-glycosylation on fungal derived recombinant products was well-known from the authors of D2 (p. 3). The fungal cells of D2 can be chosen from filamentous fungi and yeasts (p. 4, last paragraph and p. 5, lines 1-2). The modified fungal cells carry genetic modifications in at least one gene whose expression product is involved in the attachment of a mannosyl residue to the hydroxyl group of seryl or threonyl amino acids and more particularly the gene encoding the Dol-P-Man:Protein (Ser/Thr) Mannosyl Transferase : **PMT1** (p. 7, lines 1-21). The process for preparing the modified fungal cells is such that the modifications are **stable during segregation and/or non-reverting and/or non-leaky** (p. 5, lines 30-35). D2 also refers to a process for the production of recombinant products including **human s rum albumin** using such modified

fungal cells (p. 10, lines 4-10 and line 16-17). Finally, D2 exemplifies the preparation of an S. cerevisiae cell deficient in O-glycosylation activity (Example 6, p. 23-25 and example 7, p. 25-28).

The IPEA is the opinion that the process disclosed in D2 can be applied to every volume of culture medium and that the pH range disclosed in claim 1 of the present application is a standard pH range for yeast cultures. Therefore, the subject-matter of claims 1-7 can not be considered as novel or inventive in view of D2 (article 33(2) and 33(3) PCT).

Invention II :

Claims 8-52 refer to a process for purifying an albumin solution which involves a succession of chromatographic steps.

A. Novelty; article 33(2) PCT.

1. The document D1 discloses a process for the preparation of albumin which has extremely low levels of or is essentially free of colorants, metal ions, human proteins, host proteins, fragments of albumin, polymers or aggregates of albumin and viruses, and which is essentially non-glycated, relatively high in free thiol and with an intact C-terminus (Abstract, lines 1-6). The process comprises passing albumin (preferably expressed and secreted by transformed yeast) through positive mode cation exchange chromatography and then positive mode anion exchange chromatography (Abstract, lines 6-9). D1 also states that other steps like ultrafiltration, gel permeation chromatography, affinity chromatography binding the albumin using blue dyes or chromatography affinity binding contaminants like aminophenylboronic acid resin can be used (Abstract, lines 9-13). The elution of albumin with a compound having affinity for albumin, from a material having no specific affinity for albumin is also disclosed (Abstract, lines 13-15). D1 discloses the conditioning of the albumin solution with octanoate - an albumin stabiliser - to a final concentration of 1-10 mM and a pH about 4.0-5.0 (p. 3, lines 18-21).

Example 2 of D1 illustrate a process of purification of the albumin which comprises the steps of : cation exchange, affinity chromatography, ultrafiltration, gel permeation (with ultrafiltration of recycle fraction) and anion exchange. In this example, the cation exchange chromatography uses commercial cation exchange matrix such as SP-Sepharose (which comprises sulfopropyl substituents) (p. 21, first paragraph). It is also stated that the pH of the albumin solution used for cation exchange chromatography is adjusted to 4.3-4.8 (p. 16, lines 12-13) and that octanoate is added to the solution to a final concentration of 1-10 mM (p. 16, lines 9- 11). In example 2, before the step of anion exchange chromatography, the pooled recycle fraction is concentrated to a retentate concentration of 20- 120 g/L albumin (p. 24, lines 22-23). The anion exchange chromatography uses a matrix like DEAE-Spherodex or DEAE-cellulose (comprising immobilised dialkylaminoalkyl substituents as anion exchangers) (p. 25, lines 11-13)
Example 6 of D1 illustrate a variation of the process of example 2 or 4. In this example, the eluate from the cation exchange column was diluted to below 10 $mS.cm^{-1}$ (p. 34, lines 27-29) and directly loaded on the anion exchange chromatography column.
Example 3 of D1 refers to the formulation of purified albumin into a final product.

Since there is no mention in most of the claims of the present application that the anion exchange chromatography should follow directly the cation exchange chromatography, the subject-matter of claims 20, 23-28, 32, 34- 36, 41-42, 47-49 and 52 can not be considered as novel in the sense of article 33(2) PCT.

Moreover, since the step of gel permeation chromatography involve the use of a solution having a pH 5.4-5.6, this step can be considered as a process for reducing the level of nickel ions in an albumin solution according to claims 45 and 46. Therefore, claims 45 and 46 lack novelty (article 33(2) PCT).

2. The subject-matter of claims 8-19, 21-22, 29-31, 33, 37-40, 43-44 et 50-51 has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, claims 8-19, 21-22, 29-31, 33, 37-40, 43-44 et 50-51 are considered as novel in the sense of article 33(2) PCT.

B. Inventive step; article 33(3) PCT.

The document D1 is considered as the most relevant document for the evaluation of the inventiveness of claims 8-52 (see point A above for the content).

D1 also discloses the purification steps of :

(i) Positive affinity chromatography using an immobilised albumin-specific dye such as Cibacron Blue type of dye (p. 4, lines 10-19) which can be used to purify the albumin with respect to the 45 kDa N-terminal albumin fragment (p. 22 to 23).

(ii) Ultrafiltration (p.4, lines 21-31 and p. 23-24).

(iii) Gel filtration in order to purify albumin with respect to yeast antigens, pigments and dimerised albumin (p. 24).

(iv) Negative affinity chromatography with respect to albumin on a matrix with immobilised aminophenylboronate in order to remove glycoconjugates such as glycoproteins and glycolipids and poly-, oligo- and monosaccharides (Example 7, p. 35-37).

D1 states that the step involving immobilised phenylboronate may be used earlier in the process (p. 39, example 8).

The document D1 which concerns purification of recombinant human albumin discloses all the purification steps used in the present application and the result obtained after purification are similar to those obtained with the purification process disclosed in the present application, i.e. :

(i) A negligible level of glycation of the purified recombinant human albumin (p. 40, line 24-26).

(ii) A low molecular weight contaminants total peak area for albumin purified by the process of D1 which is of less than 10% of that for

human serum albumin (p. 44, lines 22-24).

(iii) Recombinant human albumin purified in accordance with the process disclosed in D1 has a stable and full length carboxy-terminus whereas HSA previously available from commercial sources appears to be heterogeneous by comparison (p. 48, lines 20-22).

(iv) A free thiol value of 0.85-0.9 mole SH/ mole rHA for albumin purified according to the process of D1 (p. 50, lines 26-27).

(v) Lower level of Al, Fe, Cu, Mg, Zn and Mn in the product of the process according to D1 than in the albumin of the prior art.

(vi) Differences in the medium and long chain fatty acid content of the albumin purified according to D1 with respect to commercial HSA (p. 57, lines 27-32 to p. 59, line 4).

(vii) Lower absorbance at 350, 403 and 500 nm than number of commercially available HSA preparations (p. 59, lines 20-21).

In view of the teaching of D1 which discloses all the important steps for the purification of human albumin used in the present application, the IPEA considers that the fact to combine the different purification steps in another order than the order disclosed in D1 or the fact to add well-known purification steps like cation or anion exchange chromatography run in negative mode with respect to albumin can not be considered as inventive. Moreover, the results of the purification process of D1 seem to be similar to the results obtained with the process of the present application. The IPEA also considers that the adjustments in the pH values, concentration and conductivity of the different solutions, which are necessary to perform the process, are current practice for the skilled person and do not imply any inventive activity.

Therefore, claims 8-44 and 47-52 can not be considered as involving an inventive step in the sense of article 33(3) PCT.

In the argumentation concerning inventive step, the applicant should demonstrate what could be the advantage of a process according to the present application over the process disclosed in the document D1.

Inv ntion III :

Claim 53 refers to a DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

Novelty; article 33(2) PCT.

The subject-matter of claim 53 has never been disclosed in the documents cited in the ISR. Therefore, claim 53 is considered as novel in the sense of article 33(2) PCT.

Inventive step; article 33(3) PCT.

The document D1 is considered as the closest prior art for the evaluation of the inventiveness of claim 53.

D1 discloses the fact that yeast may be transformed with an expression plasmid containing an expression cassette comprising a yeast promoter, a sequence encoding a secretion leader, the HSA (human serum albumin) coding sequence and a transcription terminator (p. 8, lines 14-26).

In 1999 (the present application claims the priority date of 30.01.99), it was well- known for the skilled person that, in order to reduce the problem of read-through from the ribosomes in an expression system, the number of in frame translation stop codons at the 3' end of a coding sequence could be increased.
Therefore, the IPEA considers that claim 53 does not involve any inventive step (article 33(3) PCT).

R It m VIII

Certain observations on the international application

Lack of clarity; article 6 PCT.

Inventions I, II and III :

1. Claim 1 of the present application lacks clarity for the following reasons :
   (i) The genetic modification of the cell is only defined by the fact that it causes the cell to have a reduced capacity of mannosylation, i.e. by the result to be achieved by said genetic modification.
   According to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.7 : "The area defined by the claims must be as precise as the invention allows. As a general rule, claims which attempt to define the invention, or a feature thereof, by a result to be achieved should be objected to".
   This remark also applies to claims 2-4.
   (ii) The sequences of the recombinant albumin or the recombinant albumin coding sequence are not given what renders the scope of the claim unclear.
   This remark also applies to claim 53.
   (iii) The attention of the applicant is drawn to the fact that, in the present application, the only fungal cell disclosed is a Saccharomyces cerevisiae cell mutated in the PMT1 gene. Moreover, all the fungal cells disclosed in the patent application WO 94/04687 cited in the present application are fungal cells having a mutation in a PMT gene. Therefore, the IPEA considers that the use of fungal cells having a genetic modification other than a mutation of a PMT gene is not supported by the description of the present application (article 5 PCT in combination with article 6 PCT).

2. In claim 5, the term "preferably" is used. The attention of the applicant is drawn to the fact that, according to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.6 : expressions, like "preferably", "for example", "such as" or "more particularly" should be regarded "as having no limiting effect on the scope of a claim; that is to say, the feature following any such expression should be regarded as entirely optional".

This remark also applies to claims 6, 7, 9, 10, 12, 14, 15, 24, 25, 26, 30, 31, 33-40, 42, 45, 49 and 53.

3. Claim 8 of the present application lacks clarity because there is no reference to what the "first albumin solution" should exactly be.

4. In claim 12, the use of the vague term "about" renders the scope of the claim unclear.

5. Claims 26 precise that the albumin solution has an octanoate ion concentration of 2-15 mM. The attention of the applicant is drawn to the fact that there no mention in the preceding claims that the albumin solution should contain an octanoate ion.

6. In claim 38, the compound present in the buffer used to elute the albumin from the anion exchanger is only characterized by the fact that it has a specific affinity for albumin, i.e. by the result to be achieved by said buffer, what should be avoided (see point VIII-1(i)).

7. In claim 42, It is not clear what is precisely meant by "primary separation" and "centrate conditioning".

8. In claim 48, there is no mention to which kind of "derivation" is meant what renders the scope of said claim unclear.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Articl 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
DELF/P22390PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/00257	31/01/2000	30/01/1999
Applicant		
DELTA BIOTECHNOLOGY LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   HUMAN SERUM ALBUMIN

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/00257

Box I Observations where certain claims wer found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-7

Process for producing recombinant albumin in a fungal cell containing a genetic modification that causes the cell to have at least a reduced capacity of mannosylation.

2. Claims: 8-52

Process for purifying an albumin solution which involves a series of chromatographic steps.

3. Claim : 53

DNA sequence encoding a recombinant albumin, wherein the 3' end contains two or more in-frame translation stop codons.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 16 October 2000 (16.10.00)	
International application No. PCT/GB00/00257	**Applicant's or agent's file reference** DELF/P22390PC
International filing date (day/month/year) 31 January 2000 (31.01.00)	**Priority date** (day/month/year) 30 January 1999 (30.01.99)
Applicant VAN URK, Hendrik et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

23 August 2000 (23.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Chrem
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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(19) World Intellectual Property Organization
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(10) International Publication Number
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(21) International Application Number: PCT/GB00/00257

(22) International Filing Date: 31 January 2000 (31.01.2000)

(25) Filing Language: English

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(30) Priority Data:
9902000.0 30 January 1999 (30.01.1999) GB

(71) Applicant *(for all designated States except US)*: **DELTA BIOTECHNOLOGY LIMITED** [GB/GB]; Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **VAN URK, Hendrik** [NL/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **MEAD, David, John** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **MORTON, Philip, Harvey** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **CARTWRIGHT, Andrew, John** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **CAMERON, Jason** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **BALLANCE, David, James** [GB/US]; Aventis Behring, 1020 First Avenue, P.O. Box 61501, King of Prussia, PA 19406-0901 (US). **GRANDGEORGE, Michel, Gaston, Joseph** [FR/DE]; Aventis Behring GmbH, P.O. Box 1230, D-35002 Marburg (DE). **BEREZENKO, Stephen** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **WOODROW, John, Rodney** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **SLEEP, Darrell** [GB/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB). **VERON, Jean-Luc, Bernard** [FR/GB]; Delta Biotechnology Limited, Castle Court, 59 Castle Boulevard, Nottingham NG7 1FD (GB).

(74) Agent: **BASSETT, Richard, S.**; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 5DD (GB).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
— *With international search report.*

(88) Date of publication of the international search report:
30 November 2000

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/44772 A3

(54) Title: HUMAN SERUM ALBUMIN

(57) Abstract: A process is provided for the preparation of a highly pure albumin solution the process comprising subjecting albumin (preferably expressed and secreted by transformed yeast) to a series of chromatographic steps. Preferably, the process comprises the steps of positive mode cation exchange chromatography, positive mode anion exchange chromatography, positive mode affinity chromatography, negative mode affinity chromatography (preferably using immobilised aminophenylboronic acid), negative mode cation exchange chromatography, and negative or positive mode anion exchange chromatography. A process for reducing the level of nickel in an albumin solution is also disclosed, as is a recombinant albumin coding sequence comprising two or more in-frame translation stop codons. Also disclosed is a process for producing recombinant albumin, the process comprising culturing a fungal cell expressing a recombinant albumin coding sequence, wherein the cell has a reduced capacity of mannosylation of the recombinantly-expressed albumin.

INTERNATIONAL SEARCH REPORT

Inter[illegible] Application No
PCT/GB 00/00257

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/14 C12N15/81 C07K1/18 C07K1/22 C12P21/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 31947 A (DELTA BIOTECHNOLOGY LTD ;GOODEY ANDREW ROBERT (GB); SLEEP DARRELL) 4 September 1997 (1997-09-04) claims 4,6,8,14,19 page 25, line 20 page 33, line 11 - line 12 ---	20-22, 24-27, 32, 34-36, 38, 40-42, 47-52
X	WO 94 04687 A (STRAHL BOLSINGER SABINE ;TANNER WIDMAR (DE); FLEER REINHARD (FR);) 3 March 1994 (1994-03-03) cited in the application claims 1-5,10,12,25 page 1, paragraph 3 page 10, paragraph 3 --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2000	31. 08. 00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mata Vicente, T.

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 00290 A (KABIVITRUM AB) 10 January 1991 (1991-01-10) page 2, line 5 - line 8 page 3, line 16 - line 25 example 2	45-52
X	REISS, A.L. ET AL.: "The Efficacy of Chelating Agents in Removing Nickel from Human Albumin in Vitro." NICKEL TOXICOL., PROC. INT. CONF., 2ND, 1980, pages 91-94, XP000933887 page 91, paragraph 2 -page 92, paragraph 1 page 93, paragraph 4	45,47-52
X	SARKAR, B.: "Bioinorganic Chemistry of Nickel" NATO ADV. STUDY INST. SER., SER. C, BIOENERG. THERMODYN.: MODEL SYST., vol. 55, 1980, pages 23-32, XP000933817 page 32	45,47-52
X	GOWARD, C. R. ET AL.: "Expression and purification of a truncated recombinant streptococcal protein." BIOCHEM. J. , vol. 267, 1990, pages 171-177, XP002045030 page 174, column 1, line 1 - line 7	53
A	WO 94 26873 A (KABI PHARMACIA AB ;ERNST JOACHIM (DE); JANSSON BIRGER (SE)) 24 November 1994 (1994-11-24) page 3, line 15 - line 31	1-7
A	US 5 284 777 A (ROSENTHAL MURRAY A ET AL) 8 February 1994 (1994-02-08) column 2, line 31 - line 38 column 3, line 1 - line 24	8-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/00257

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

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Remark on Protest

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☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/GB 00/00257

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